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Development and Validation of RP-HPLC Method for Estimation of Nebivolol and Indapamide in Bulk Drug and Formulation

Rajendra D. Dighe[1], Chandraprakash R. Deore[2]*, Sadhana B. Mahajan[3], Vinod A. Bairagi[4]

[1,2]Department of Pharmaceutical Quality Assurance, K.B.H.S.S. Trust's Institute of Pharmacy Malegaon, Nashik- 423105, Maharashtra, India

[3]Department of Pharmaceutical Chemistry, K.B.H.S.S. Trust's Institute of Pharmacy Malegaon, Nashik-423105, Maharashtra, India

[4]Department of Pharmacology, K.B.H.S.S. Trust's Institute of Pharmacy Malegaon, Nashik-423105, Maharashtra, India

***Corresponding Author - deorechandra143@gmail.com**

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ABSTRACT

The research presents the development and validation of a robust RP-HPLC (Reversed-Phase High-Performance Liquid Chromatography) method for the simultaneous estimation of Indapamide and Nebivolol in pharmaceutical formulations. Utilizing an Agilent system with an auto sampler, Gradient System DAD Detector, and a C_{18} column, the method employed a mobile phase consisting of 35% Methanol and 65% water with 0.1% TFA. The detection was performed at a wavelength of 257 nm with a flow rate of 1.1 ml/min, resulting in retention times of 5.316 minutes for Indapamide and 7.467 minutes for Nebivolol. Method validation was conducted following ICH guidelines, covering parameters such as linearity, precision, range, and robustness. The results confirmed that the method is simple, accurate, precise, economical, and replicable. This newly developed RP-HPLC method proves to be suitable for routine quality control analysis of Indapamide and Nebivolol in bulk drugs and their formulations, ensuring no interference during simultaneous determination.

Keywords: Nebivolol, Indapamide, RP-HPLC, Method Validation, etc.

Introduction

Analytical chemistry is a pivotal field focused on the quantitative examination of the composition of substances and complex materials across diverse matrices[1].This examination is accomplished by quantifying a physical or chemical characteristic of a distinguishing constituent of the components of interest. Analytical procedures are categorized based on the attribute of the analyte being measured, and one of the most significant applications of this field is pharmaceutical analysis[1].

The proliferation of analytical instruments today has resulted in what may seem like a confusing array of acronyms, yet these tools collectively contribute to a Holistic field [1].The precision of analytical procedures should be tailored to be as necessary for the purpose, rather than as feasible. Analytical methods are broadly classified into instrumental and chemical methods. Instrumental methods encompass dimensions such as light wavelength and intensity, fluorescence, resistivity, and electrode polarity. Chemical methods, on the other hand, involve measurement using gravimetric or volumetric approaches to determine the mass of the analyte [3].

The importance of analytical techniques cannot be overstated, as they are crucial for maintaining and ensuring high substance standards, serving as key components of quality assurance and quality control. In the realm of pharmaceutical analysis, a variety of instrumental approaches are employed to achieve these goals [4].

Pharmaceutical analysis is an essential component of pharmaceutical sciences. Within this domain, research analysts bear significant responsibilities, as outlined by [4].These responsibilities include:

- Developing analytical techniques for the product's components including raw materials, active ingredients, and chemical intermediates [5].
- Creating methods for the selective analysis of drugs, Formulation ingredients, breakdown products, and impurities, as well as identifying degradation products, degradation pathways, and the degree of deterioration under various storage conditions[8].
- Establishing analytical methods for micro and semi-micro quantities of drugs and their metabolites in biological systems[11].

This paper aims to explore the diverse analytical techniques employed in pharmaceutical analysis, highlighting the critical role they play in ensuring the quality and efficacy of pharmaceutical products [12,13].The discussion will encompass separation techniques, spectrometric techniques, and other relevant analytical methodologies, providing a comprehensive overview of current practices and advancements in the field [15]

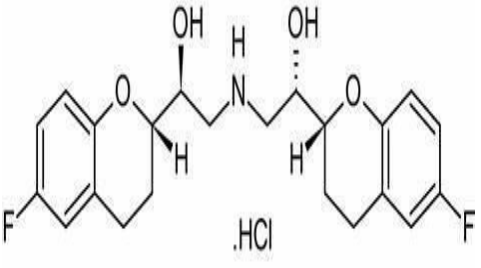

Fig.No.1. Structure of Nebivolol

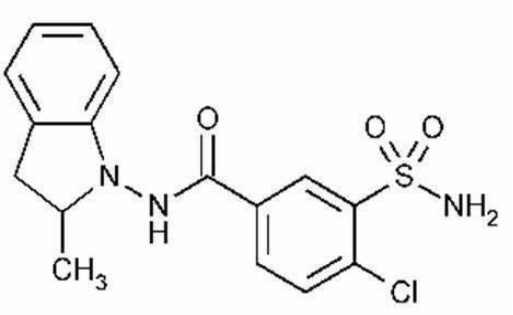

Fig.No.2. Structure of Indapamide

Chromatographic behavior of Indapamide and Nebivolol

Table No.1: Chromatographic Behavior of Indapamide and Nebivolol Mobile phase of Various Compositions.

Sr No.	Mobile Phase	Retention Time (min)		Remark
		IDP	NBV	
1.	Methanol+ Water TFA 0.1 %(80:20 % v/v) 0.7ml 257 nm	1.785	-	Merge peak Observed
2	Methanol+ Water TFA 0.1%(70:30 % v/v) 0.7ml 257 nm	1.705	1.837	No Sharpe peak
3	Methanol + Water TFA 0.1%(60:40 % v/v) 0.7 ml 257 nm	1.760	1.935	No Sharpe peak
4	Methanol + Water TFA 0.1%(50:50 % v/v) 0.7 ml 257 nm	1.740	1.913	No Sharpe peak
5	Methanol + Water TFA 0.1%(35:65 % v/v) 0.7 ml 257 nm	5.523	7.874	Sharpe peak

Materials and Methods

Instrumentation and Software:

HPLC Agilent Tech. Gradient System with Auto injector

Software: Chemstation

Materials and Reagent:

Nebivolol, Indapamide, Orthophopsphoric acid (OPA), Methanol, Water.

Preparation of Solutions:

Preparation of std. Indapamide solution: (Stock I)

Take 0.1–0.5 mL of the freshly prepared standard stock solution (150 µg/mL) and transfer it to a 10 mL volumetric flask. Fill up to the 10 mL mark with the mobile phase to obtain a final concentration of 1.5–7.5 µg/mL [18].

Preparation of std. Nebivolol solution: (Stock II)

Take 0.1–0.5 mL of the freshly prepared standard stock solution (500 µg/mL) and add it to a 10 mL volumetric flask. Adjust the volume to 10 mL with the mobile phase to achieve a final concentration of 5–25 µg/mL [18].

Preparation of std. Indapamide and Nebivolol solution :(Stock III)

Take 0.1–0.5 mL from the freshly prepared standard stock solutions of Indapamide (5 µg/mL) and Nebivolol (25 µg/mL) and transfer to separate 10 mL volumetric flasks. Dilute to the final volume with the mobile phase to achieve a concentration of
1.5–7.5 µg/mL for Indapamide and 5–25 µg/mL for Nebivolol [18].

Selection of mobile phase

The mobile phases underwent vacuum degassing and were filtered through a 0.45 µm membrane filter. Following this, the mobile phase was allowed to equilibrate until a stable TFA baseline was achieved. A series of tests with various individual solvents and solvent combinations were conducted using a standard solution of Indapamide and Nebivolol to determine the best conditions for separation and stable peak formation. The Methanol and Water (0.1% TFA) mobile phase was selected due to its effectiveness in producing sharp, well-resolved peaks with appropriate symmetry and reproducible retention times for Indapamide and Nebivolol [19]. The chromatograms are shown in Table No: 1 [20,21].

Result and DiscussionsPreliminary trials:

The observed initial trials using methanol and 0.1% TFA in the ratio of 35:65 at 257nm. As mention in table 1 trial 1 to 5 where unaccepted due to several reasons and the accepted trial with obtained sharp peak was 5.

Table No.2: Preliminary Trials

Sr. No.	Column used	Mobile phase ratio	Detection wavelength	Flow rate	Injection volume	Run time	Result	Conclusion
1	Agilent C-18(150mm x 4.6mm, 5µm)	Methanol with 0.1% TFA (80:20)	257 nm	0.7 ml/min	20 µl	8 min	Single peak was observed	Method unaccepted
2	Agilent C-18(150mm x 4.6mm, 5µm)	Methanol with 0.1% TFA (70:30)	257 nm	0.7 ml/min	20 µl	8 min	Merge peak was observed	Method unaccepted
3	Agilent C-18 (150mm x 4.6mm, 5µm)	Methanol with 0.1% TFA (60:40)	257 nm	0.7 ml/min	20 µl	8 min	Peak separation was not observed	Method unaccepted
4	Agilent C-18(150mm x 4.6mm, 5µm)	Methanol with 0.1% TFA (50:50)	257 nm	0.7 ml/min	20 µl	8 min	Peak separation was not observed	Method unaccepted
5	Agilent C-18(150mm x 4.6mm, 5µm)	Methanol with 0.1% TFA (35:65)	257 nm	1 ml/min	20 µl	10 min	Separation and Sharpe peak were obtained	Method accepted

Method development

The validation of the method adhered to the guidance of the ICH. Various validation parameters were assessed; including linearity, accuracy, precise and its limit of detect and quantify [21].

Optimized chromatographical conditions

Optimized chromatographical conditions and typical chromatogram were presented in Table 3 and Figure 3.

Table.No.3: The final chromatographic conditions selected were as follow:

Parameters	**Conditions**
Instrumentation	Water HPLC with auto sampler and DAD detector
Injected volume	20µl
Mobility phase	Methanol: 0.1% TFA (35:65)
Column	Agilent C18 (150 mm x 4.6 mm, 2.5 µm)
Flow rate	1ml/min
Run time	15 minutes
Separation mode	Isocratic mode

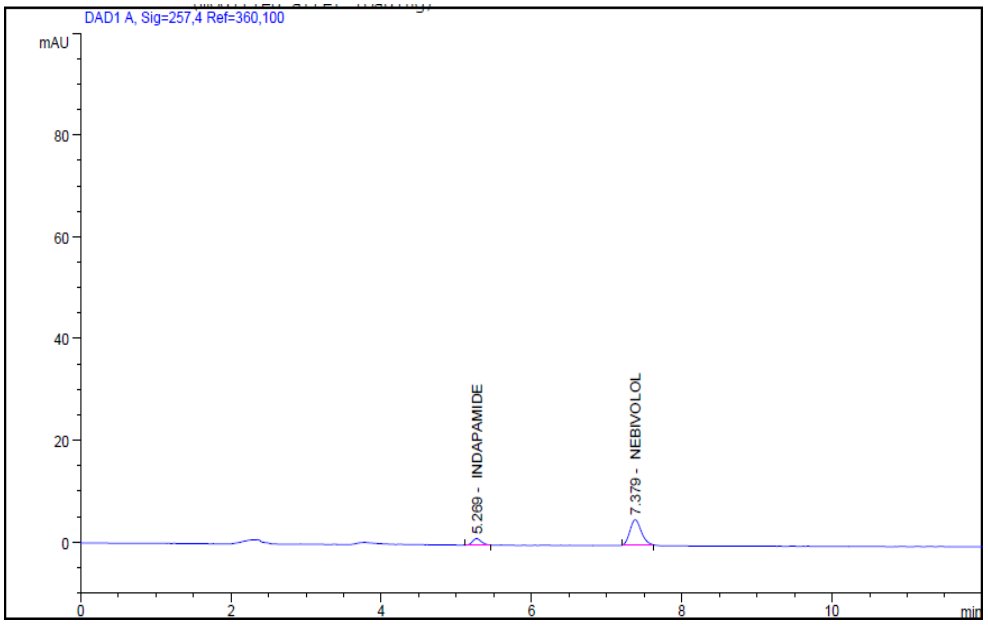

Figure No.3: Chromatogram of standard Combination of Indapamide and Nebivolol.

Calibration experiment

RP-HPLC Method :

Analysis of the calibration data via linear regression revealed a linear correlation between the peak areas and concentrations for Indapamide (1.5–7.5 µg/mL) and Nebivolol (5–25 µg/mL). The calibration data are shown in Table No: 3 for Indapamide and Table No: 4 for Nebivolol. The obtained linear equations were y = 7.286x - 0.441 for Indapamide and y = 10.58x + 0.085 for Nebivolol, where x is the concentration and y is the peak area. The correlation coefficient for both analytes was 0.999. The calibration curves for both compounds are depicted in **Fig No: 3 and Fig No: 4.**

Table No.3 : Linearity data for Indapamide

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	1.5	10.241	10.7136	10.4773	0.3342	3.1895
	3	21.1586	21.0293	21.0940	0.0914	0.4334
	4.5	32.0412	33.6466	32.8439	1.1352	3.4563
	6	43.3752	43.2435	43.3093	0.0931	0.2150
	7.5	54.0414	54.0005	54.0210	0.0289	0.0535
	Equation		y = 7.286X+0.441			
	R^2		0.999			

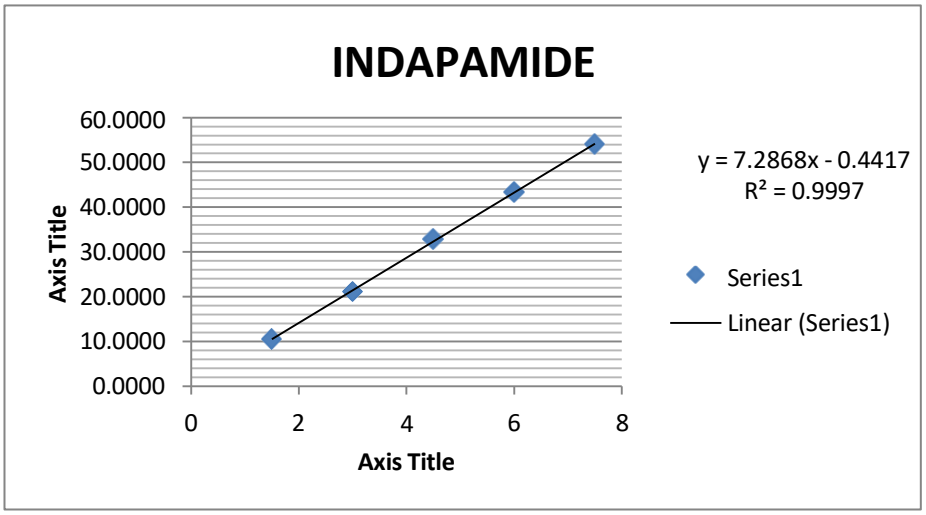

Fig.No.4 : Calibration curve of Indapamide

The RP-HPLC method yielded the linear equation y = 7.286X + 0.441 for Indapamide, where x is the concentration and y is the peak area. The correlation coefficient for this relationship was 0.999. The calibration curve for Indapamide is shown in Fig. No. 4.

Table No.4 : Linearity data for Nebivolol

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	5	53.3366	51.616	52.4763	1.2166	2.3185
	10	105.4405	105.8331	105.6368	0.2776	0.2628
	15	155.5924	159.8164	157.7044	2.9868	1.8939
	20	211.0795	210.7351	210.9073	0.2435	0.1155
	25	264.5112	264.3617	264.4365	0.1057	0.0400
	Equation		y = 10.58x + 0.085			
	R^2		0.999			

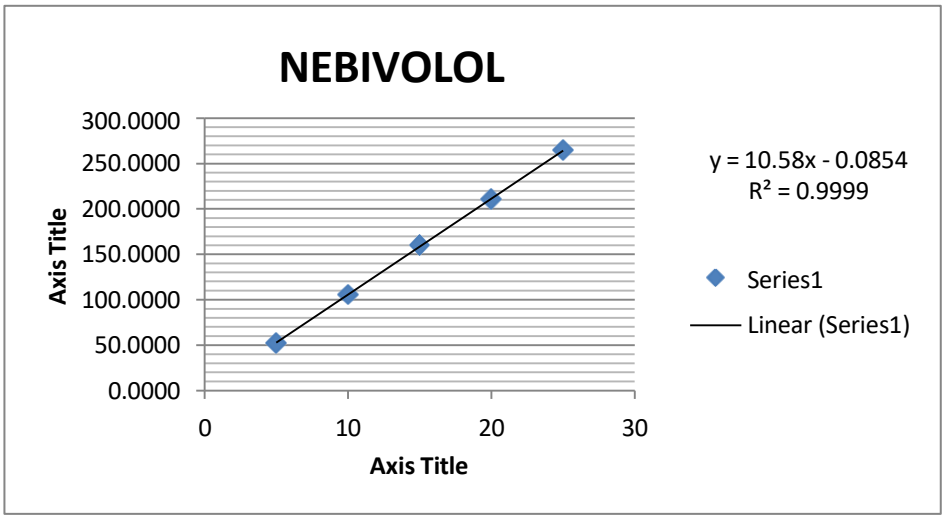

Fig.No.5 : Calibration curve of Nebivolol

The RP-HPLC method yielded a linear equation for Nebivolol as y=10.58x+0.085y = 10.58x + 0.085y=10.58x+0.085, where xxx represents the concentration and yyy denotes the peak area. The correlation coefficient was found to be 0.999, and the calibration curve for Nebivolol is shown in **Figure 5.**

Analytical of Method Validation:

1. Linearity:

From the Indapamide standard stock solution, various working standard solutions (1.5-7.5 µg/ml) were prepared in the mobile phase. Similarly, from the Nebivolol standard stock solution, different working standard solutions (5-25 µg/ml) were prepared in the mobile phase. A 20 µl sample of each solution was injected into the chromatographic system using a fixed volume loop injector, and chromatograms were recorded. The area for each concentration was documented **(Table.5).** The calibration curves are depicted in **Figures 6 and 7.**

Table No.5: Linearity of Indapamide

Concentration µg/ml	**Area Indapamide**
1.5	10.4773
3	21.0940
4.5	32.8439
6	43.3093
7.5	54.0210

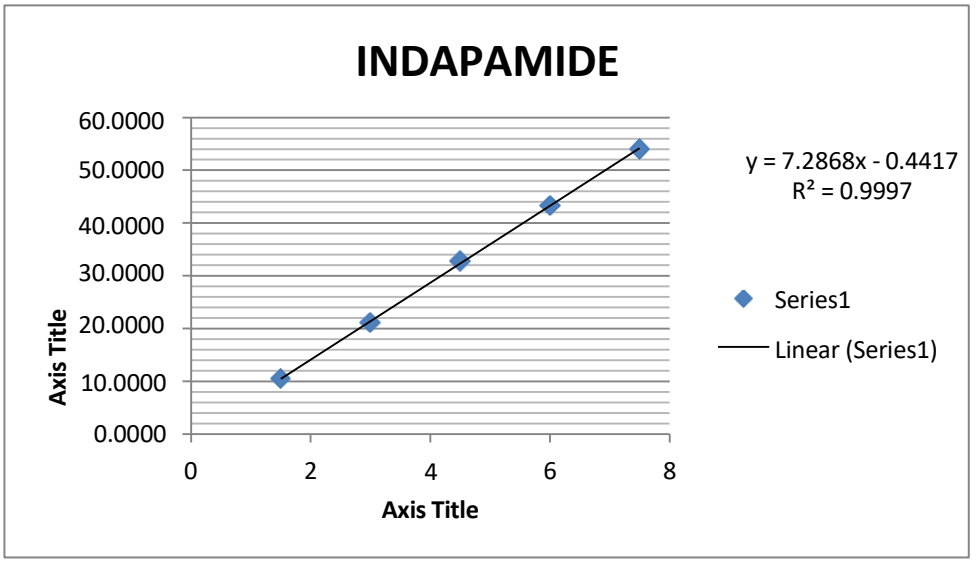

Fig No.6 : Calibration graph of Nebivolol for HPLC method

Table No.8 : Regression equation data for Nebivolol

Regression Equation Data Y=mx+c	
Slope(m)	10.58 X
Intercept(c)	0.085
Correlation Coefficient	0.999

The linearity of Indapamide and Nebivolol was observed in the concentration ranges of 1.5-7.5 µg/ml and 5-25 µg/ml, respectively. The detection wavelength used was 257 nm (Tables 7 and 8). The plot should be linear and pass through the origin, with the correlation coefficient not being less than 0.999, as concluded in **Table 6.**

2. Accuracy:

Recovery studies were conducted to validate the accuracy of the developed method. To a pre-analyzed tablet solution, a known concentration of standard drug (80%, 100%, and 120%) was added, and its recovery was then analyzed **(Table 8).** Statistical validation of the recovery studies is shown in **Table 9.**

Table No.8: Result of Recovery data for Indapamide and Nebivolol

METHOD	**Drug**	**Level (%)**	**Amt. taken (µg/m l**	**Amt. Added (µg/ml**	**area Mean* ± S.D.**	**Amt. recovered Mean *±S.D.**	**%Recovery Mean *± S.D.**
RP-HPLC Method	**IDP**	80%	1.5	1.2	2.6±0.0064	1.19±0.006	99.35±0.52
		100%	1.5	1.5	3.00±0.002	1.5±0.0028	100.13±0.18
		120%	1.5	1.8	3.32±0.0068	1.81±0.006	101.14±0.37
	NBV	80%	5	4	8.98±0.001	3.98±0.001	99.74±0.030
		100%	5	5	9.97±0.0001	4.97±0.001	99.4839±0.02
		120%	5	6	10.96±0.016	5.96±0.001	99.50±0.32

***mean of each 3 reading for RP-HPLC method**

Table No.9 : Statistical Validation of Recovery Studies Indapamide and Nebivolol

METHOD	Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
Rp-HPLC Method	80%	IDP	99.35	0.5221	0.52
		NBV	99.74	0.03	0.030
	100%	IDP	100.13	0.18	0.183
		NBV	99.48	0.002	0.002
	120%	IDP	101.14	0.374	0.3705
		NBV	99.50	0.322	0.3240

***Denotes average of three determinations for RP-HPLC.**

The accuracy of the RP-HPLC method was confirmed through recovery studies performed at different concentration levels (80%, 100%, and 120%). The percentage recovery was found to be within the range of 98-102% **(Table 8).**

3. System Suitability Parameters (Repeatability):

To ensure the resolution and reproducibility of the proposed chromatographic system for the estimation of Indapamide and Nebivolol, system suitability parameters were evaluated. The results are presented in **Table 10.**

Table No.10: Repeatability studies on RP-HPLC for Indapamide and Nebivolol

Sr.No.	Concentration of IDP (mg/ml)	Peak area	Amount found (mg)	% Amount found
1	6	43.47441	43.30	100.040
2	6	43.1161		
		Mean	43.30	
		SD	0.2534	
		%RSD	0.169	

Sr.No.	Concentration of NBV (mg/ml)	Peak area	Amount found (mg)	% Amount found
1	3	209.06781	210.48	99.80
2	3	211.8890		
		Mean	210.48	
		SD	1.99	
		%RSD	0.16	

Repeatability studies on RP-HPLC method for Indapamide and Nebivolol was found to be,The %RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded .**(Table No.10)**

4. Precision:

The method was established by analyzing various replicates standards of Indapamide and Nebivolol. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in **(Table No.11)** respectively.

Table No. 11: Result of Intraday and Inter day Precision studies on RP-HPLC for Indapamide and Nebivolol.

METHOD	Drug	Conc^{n} (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
Rp-HPLC METHOD	IDP	3	21.68± 0.96	102.10	21.42±5.65	100.9
		4.5	32.17±0.11	99.92	32.35±0.28	100.54
		6	43.25±0.54	100.75	43.01±0.14	99.61
	NBV	10	105.55± 0.96	100.25	105.44±5.65	100.11
		15	161.51±0.80	102.16	161.02±2.73	101.85
		20	209.37±0.17	100.00	210.92±1.87	100.01

***Mean of each 3 reading for RP-HPLC method**

Intraday and interday precision studies on the RP-HPLC method for Indapamide and Nebivolol demonstrated high precision, with % amounts ranging between 98% and 102%. This indicates that the analytical method is reliable **Table 11.**

5. Robustness:

The robustness of the method refers to its ability to remain unaffected by small deliberate changes in experimental conditions. To evaluate the robustness of the proposed method, deliberate variations were made in the optimized method parameters, including changes in mobile phase composition, flow rate, and wavelength. The effects of these changes on the retention time and tailing factor of the drug peak were studied by varying the mobile phase composition (±1 ml/min), flow rate (±1 ml/min), and wavelength (±1 nm) from the optimized chromatographic conditions. The results of the robustness studies are shown in **(Tables 12,13).** The robustness parameters were found to be satisfactory, confirming the reliability of the analytical method.

Table No.12: Result of Robustness Study of Indapamide

Parameters	**Conc.(µg/ ml)**	**Amount of detected(mean ±SD)**	**%RSD**
Chromatogram of flow change 0.9 ml	7.5	54.55±0.45	0.83
Chromatogram of flow change 1.1 ml	7.5	56.91±0.30	0.53
Chromatogram of comp change 34 Methanol +66 Water	7.5	71.0±0.13	0.18
Chromatogram of comp change 36 Methanol+ 64 Water	7.5	72.50±0.49	0.68
Chromatogram of wavelength 256nm	7.5	47.3±0.08	0.16
Chromatogram of wavelength 258nm	7.5	56.94±0.10	0.17

6. Robustness Study of Indapamide:

In the robustness study for Indapamide, changes were made to the flow rate (±1 ml/min) and pH of the mobile phase composition. The %RSD for the peak area was calculated and found to be less than 2%, indicating that the method is robust. The results are presented in Table 12, confirming the reliability of the analytical method.

Table No.13: Result of Robustness Study of Nebivolol

Parameters	**Conc.(µg/ml)**	**Amount of detected(mean ±SD)**	**%RSD**
Chromatogram of flow change 0.9 ml	25	225.97±0.36	0.16
Chromatogram of flow change 1.1 ml	25	250.79±1.13	0.45
Chromatogram of comp change 34 MEOH +66 WATER	25	297.4±1.28	0.43
Chromatogram of comp change 36 Methanol+ 64 WATER	25	273.13±0.64	0.23
Chromatogram of wavelength 256nm	25	238.9±1.92	0.81
Chromatogram of wavelength 258nm	25	281.96±0.39	0.14

7. Robustness Study of Nebivolol:

In the robustness study, variations were introduced in the flow rate (±1 ml/min) and pH of the mobile phase composition (±1 unit). The %RSD for peak area was calculated and found to be less than 2%, indicating that the analytical method is robust. These results are summarized in **(Table 57),** affirming the reliability of the method.

Limit Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope The limit of detection (LOD) may be expressed as:

LOD = 3.3 (SD)/S

Where, SD = Standard deviation of Y intercept S = Slope

Limit of detection = 3.3 X 0.13/7.320= 0.0586 (µg/mL)

Limit of Quantitation = 10 X 0.13/7.320= 0.1775 (µg/mL)

The LOD and LOQ of Indapamide was found to be 0.0586 (µg/mL) and 0.1775 (µg/mL), analytical method that concluded.

Limit Quantification

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,
The quantitation limit (LOQ) may be expressed as:

LOQ = 10 (SD)/ S

Where, SD = Standard deviation Y intercept
S = Slope

Limit of Detection = 3.3X0.82/10.56=0.2582 (µg/mL)

Limit of Quantitation = 10 X0.82/ 10.56=0.7765 (µg/mL)

The LOD and LOQ of Nebivolol was found to be **0.2582**(µg/mL) and **0.7765**(µg/mL), analytical method that concluded.

Analysis of tablet formulation:

Procedure:

Weigh a combination of Indapamide and Nebivolol and calculate the average weight. Accurately weigh and transfer a sample equivalent to 1.5 mg Indapamide and 5 mg Nebivolol into a 10 ml volumetric flask. Add approximately 10 ml of methanol as the diluent, sonicate to completely dissolve the sample, and make up the volume to the mark with the diluent. Mix well and filter through a 0.45 µm filter. Then, pipette 0.4 ml of this stock solution into a 10 ml volumetric flask and dilute to the mark with diluent to obtain a final concentration of 4.5 + 15 µg/ml. The chromatograms for the test solution of Indapamide and Nebivolol are shown in Figures 10 and 11. The amounts of Indapamide and Nebivolol per drop were determined by extrapolating the peak areas from the calibration curves. The analysis procedure was repeated five times with the tablet formulation, and the %RSD for the % label claim was calculated. The results are presented in **Table 14.**

Brand Name: Nebula-D

Total weight of 20 Tab Powder wt. = 0.389 gms

Avg Powder Weight = 19.47 gms./Tab Eq.Wt for 5 mg= 5 x 19.47/ 5 = 19.47 mg
Take 19.47 mgs in 10 ml Methanol i.e= 150+500 µgm/ml

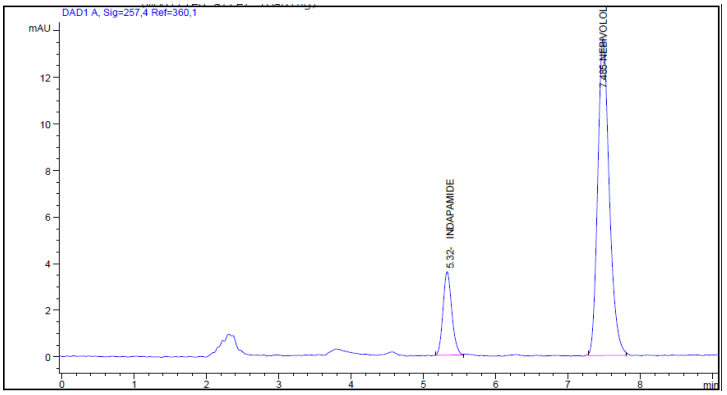

Fig No.9: Chromatogram for Marketed Formulation.

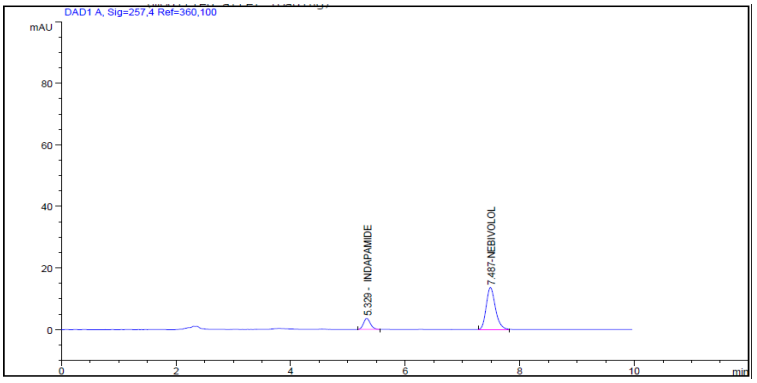

Fig No.10: Chromatogram for Marketed Formulation.

Table No.14: Analysis of marketed formulation.

Aassy	Drug	conc	Amt.Found	%Lable Claim	SD	%RSD
Rp- HPLC Method	IDP	4.5	32.4338	100.7128	0.041	0.1694
	NBV	15	14.9655	99.7670	0.9405	0.9473
	IDP	4.5	32.4501	100.7622	0.0412	0.0169
	NBV	15	14.9729	99.8194	0.940	0.947

The analysis of the marketed formulation showed % label claim values within the range of 98-102%, which is considered satisfactory. This conclusion is presented in Table 14.

8. Ruggedness:

Ruggedness refers to the degree of reproducibility of test results obtained from the analysis of the same sample under various conditions, such as different analysts, laboratories, or instruments.

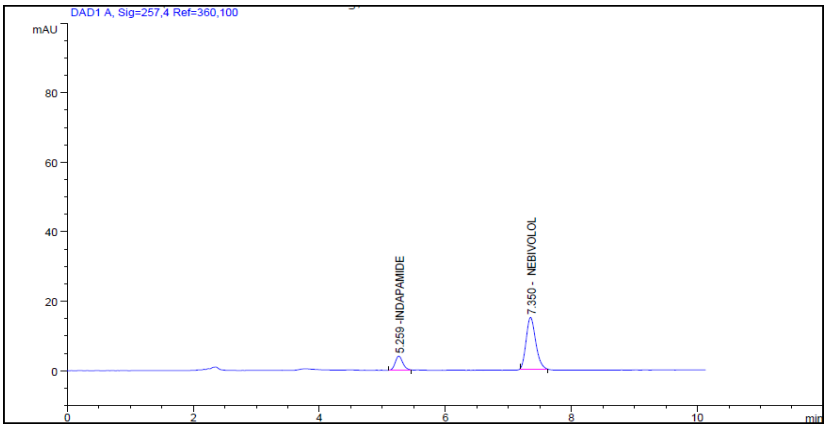

Fig No.11: Chromatogram for Analyst-1 (4.5+15 mcg)

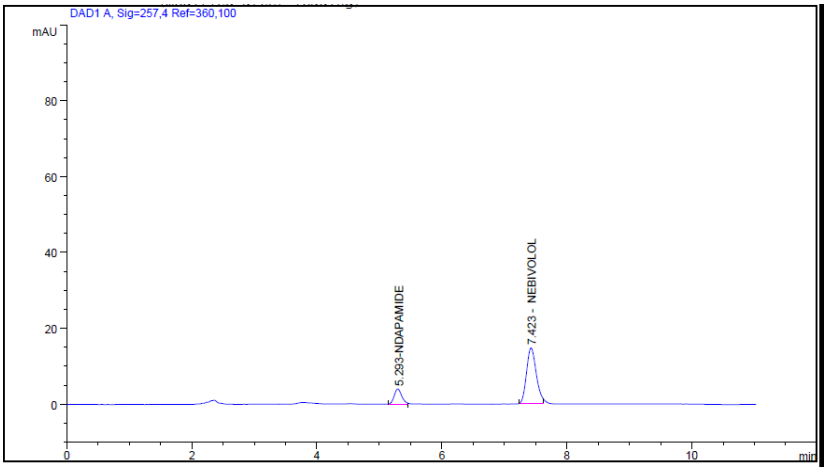

Fig No.12: Chromatogram for Analyst-II (4.5+15 mcg)

Table.No.15: Analysis of Analyst-1 (4.5+15 mcg)

Drug name	**R.T**	**AREA**	**TH.PLATES**	**SYMM**
Analyst-I	5.263	32.8954	8963	0.82
(IDP)	5.293	32.0012	9452	0.83
Analyst-II	7.340	155.89562	10236	0.79
(NBV)	7.423	160.8963	10563	0.83

Conclusion

A simple, rapid, accurate, and precise RP-HPLC method, along with a spectrophotometric method, has been developed and validated for the routine analysis of Indapamide and Nebivolol in both API and formulation. Both methods are effective for the simultaneous determination of Indapamide and Nebivolol in multi-component formulations without interference. These developed methods are recommended for routine and quality control analysis of these drugs in two-component pharmaceutical preparations. The results obtained

from the proposed methods were in excellent agreement with the label claims of the formulations. Additionally, the standard deviation and coefficient of variation values were satisfactorily low, demonstrating the methods' suitability for routine estimation of tablet dosage forms.

References:

1. Anjaneyules Y., Chandrasekhar K.A text book of Analytical Chemistry, 1st ed. Publisher ministry of defence, defence research and development organization, recruitment and organization centre, Lukhnow Road, Timarpur Delhi, 2006: p.1.
2. Rashmin. An introduction to analytical method development for pharmaceutical formulations. Pharma info.net, 2008: 6(4): p.1.
3. Sethi. PD. In; HPTLC Quantitative analysis of pharmaceutical formulations, CBS publishers and distributors, New Delhi, 2001: 1st ed, preface IIV, p.3.
4. Douglas AS., Holler FJ., Crouch SR. Principle of Instrumental Analysis, Thomson Publication, 2007: 6th ed, p.1.
5. Sharma BK., Instrumental Methods of Chemical Analysis, Goel Publishing House, Meerut, 2002: 23rd ed, p.7-8.
6. Kalra K. Method Development and Validation of Analytical Procedures, Quality Control of Herbal Medicines and Related Areas, Prof. Yukihiro Shoyama (Ed.),2011: 307(9),p.1-5
7. Beckette AH, Stenlake GH. Practical Pharmaceutical Chemistry. CBS Publishers and Distributors, New Delhi, 2004:4(2) p.1.
8. Jeffery GH. Besset J. Mendham J. Denney RC. Vogel's Text Book of Quantitative Chemical Analysis. Longman Scientific and Technicals. 1999, 5th ed,p.3.
9. Sharma BK. Instrumental Methods of Chemical Analysis, Meerut : Goel Publishing House; 2004: 23rd ed, P.7-8.
10. Skoog DA, West DM, Holler FJ. Analytical chemistry – An Introduction, 6th ed. Saunder college Publishing. p.3.
11. Quality Assurance of Pharmaceuticals. A compendium of guidelines and related materials. Universal Publishing Corporation, Mumbai, 1999.(1) p. 119-123.
12. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental Methods of Analysis. A 7th ed. CBS Publisher and Distributors, New Delhi.p.5.
13. Kasture AV, Wadodkar SG, Mahadik KR, More HM. A Textbook of Pharmaceutical Analysis.Nirali Prakashan, 2004:2(10.ed) p.4,513
14. Sethi PD. High Performance Liquid Chromatography Quantitative Analysis of Pharmaceutical New Delhi: CBC Publication and Distributors; 2001:p.8-10
15. Skoog D, Holler F, Nieman T. Principles of Instrumental Analysis.Thomson Books; 2006. 5th ed. New Delhi p.11-16.
16. Cindy Green, RAC. A Step By Step Approach to Establishing a Method Validation. Journal of Validation Technology August 2007; 13(4):p.317-323.

17. Vyas, N., & Panchal, S. (2014). Development and validation of RP-HPLC method for simultaneous estimation of nebivolol and indapamide in pharmaceutical dosage form. *Asian Journal of Pharmaceutical Analysis*, *4*(3), 98-102.
18. Praveena, T., & Parameswari, S. A. (2018). A modified RP-HPLC method development and validation for the simultaneous estimation of nebivolol and indapamide in bulk and pharmaceutical dosage forms. *world j pharm res*, *7*(15), 860-73.
19. Gaur, B., Gaur, K., Sekhawat, K. S., & Singh, A. (2013). Development and Validation of High Performance Liquid Chromatography Method for Simultaneous Estimation of Nebivolol and Indapamide in Their Combined Tablet Dosage Form. *World Journal of Chemistry*, *8*(1), 11-19.
20. Chodavadia, R. M., Marolia, B. P., Shah, S. A., & Dudhat, G. C. (2012). First order derivative spectrophotometric method for simultaneous estimation of nebivolol hydrochloride and indapamide in tablet dosage form. *Asian Journal of Research in Chemistry*, *5*(2), 282-285.